INDEPENDENCE OF POST-FERTILIZATION RESPIRATION IN THE SEA-URCHIN EGG FROM THE LEVEL OF RESPIRATION BEFORE FERTILIZATION

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Introduction

The unfertilized sea-urchin egg just removed from the ovary respires at an oxygen consumption rate comparable with that of the newly fertilized egg (Borei, 1948a). After a few hours the respiration gradually drops, however, to a low, fairly constant level. This level represents the low respiration value of the unfertilized sea-urchin egg previously recorded in the literature. From this level there is a sudden jump at fertilization to the respiration rate of the newly fertilized egg. In Psammechinus the rate of the latter is usually three to four times that of the unfertilized egg at the constant level. For further details see Borei (1948a).

Now the question arises whether the increase in respiration after fertilization is merely an addition to the pre-fertilization respiration, or whether it competes with the existing respiration, eventually suppressing it completely.

In view of the concept (Runnström, 1930, Robbie, 1946) that the oxidase system in operation in the egg is the same before and after fertilization, and if parallels to events in the Asterias egg are considered (Borei and Lybing, 1949), the addition possibility is not unlikely. The difficulties arising in trying to interpret in this manner results from cyanide-inhibition experiments on fertilized eggs (Korr, 1937) could be avoided if Lindahl's (1940) findings are taken into account. He states that the cyanide-stable respiration arises under the influence of the inhibitor, and his view is strongly supported by Robbie's (1946) experiments.

On the other hand, Ballentine's (1940) concept that at fertilization a link in the dehydrogenase part of the oxidative chain is introduced, thus inducing an augmented respiration, offers a possibility that post-fertilization respiration, competing with that of pre-fertilization, may successfully suppress the latter, in spite of the fact (Robbie, 1946) that the same oxidase system is in operation both before and after fertilization. Korr, who first (1937) favored the view that cytochrome c is the link put into operation at fertilization, later (1939) abandons this and also turns to a concept that new ultimate substrate is released from precursors.

Runnström's (1930, 1932 and 1935) view that the oxidase is unsaturated with its substrate in the unfertilized egg, is equally consistent with both possibilities.

Borei and Lybing (1949) find that results from experiments on the temperature characteristics of sea-urchin eggs before and after fertilization can not decide between the two possibilities. It was stated by them that all facts at present known about sea-urchin egg respiration still leave the question open: It is just as possible to assume a simple addition as to believe in a competition.

It was thought that measurements of post-fertilization respiration in such experiments, where the eggs were fertilized at times corresponding to different points

on the decreasing respiration curve of the unfertilized egg, would help to elucidate the matter. If the rate of oxygen consumption before fertilization influenced the respiration of the fertilized egg, the addition possibility would have to be strongly considered. If not, one would be inclined to think that post-fertilization respiration is, as a whole, different from that before fertilization.

For a fuller discussion of previous literature on the biochemical aspects concerning sea-urchin egg respiration changes at fertilization, see Borei and Lybing (1949).

Material and Methods

The egg respiration of *Psammechinus miliaris* has recently (Borei, 1948a, Borei and Lybing, 1949) been studied in some detail. This species (phenotype S) was therefore chosen also in this investigation. For particulars concerning the material see Borei (1948a), Chapters 2.1, 3.111 and 3.21.

In order to make measurements possible on several lots of eggs taken from the same female and fertilized at subsequent times from the moment of removal from the ovary, one must use a method on the μl. scale. Thus Cartesian diver micro-respiration technique was employed. Concerning technical points see Borei (1948a), Chapters 2.2 and 2.3. Diver charge Type I (Borei, 1948b) was used throughout.

All experiments were performed at 18° C. After completed diver measurements, the cells were taken out of the divers and observed as to condition and fertilizability. The only experiments accepted were those in which these controls turned out satisfactorily.

In the actual runs, the procedure was as follows: Eggs were removed from the ovary, a lot was immediately fertilized, then a diver was charged with unfertilized eggs and these were brought to measurement as rapidly as possible. (The first respiration values could thus be obtained $\sim$20 mins. after egg removal from ovary.) This control diver was then continuously followed during all subsequent measurements on fertilized eggs from the same animal. From the first fertilized lot a diver was now charged. Subsequently new lots were fertilized and corresponding divers charged. In the experiments with fertilized eggs, the respiration rate at 120 minutes after fertilization, where the exponentially increasing respiration curve is still rather flat (see Borei, 1948a, Figure 3), was estimated and used for comparison. For unfertilized eggs a number of about 100, and for fertilized about 50, were found to be most suitable for charging the divers, which were of approximately 7 μl. capacity.

Results and Interpretations

The results from experiments on eggs from three females are represented in Figure 1.

It is obvious that respiration after fertilization reaches exactly the same level, irrespective of the height of the prevailing respiration at the moment of fertilization. It is of no importance whether the fertilization sets in at a very early moment, when the egg has just been removed from the ovary and accordingly respires at a very high rate, or whether it is effected a very long time after the removal, when the egg has alrady reached a fairly constant, low-rate respiration. In both cases the post-fertilization respiration will be the same.

In this connection it must be pointed out that the respiration of the fertilized egg in this investigation has been followed until more than nine hours after removal of the eggs from the ovary. This is considerably longer than in the cases reported by Borei (1948a), where the measurements were discontinued after about six hours. In no case, however, has there been observed any rise in the respiration rate at the end of the experiments, as might have been expected in consideration of the early findings of Warburg (1914) and Runnström (1928). Goldforb (referred to in Gerard and Rubenstein, 1934) found that the increase begins about five hours after shedding, and Tyler and Humason (1937) report a steady increase over the

whole measurement period. Runnström (1928) thinks that the increase is an indication of the "aging" of the egg (cf., however, Borei, 1948a, Chapter 3.113, who finds that over-ripe eggs have lower respiration than ripe ones and still lower than under-ripe ones). Tyler, Ricci and Horowitz (1938) found that the increase is avoided if the experiments are carried out under sterile conditions. It must be stated that all previous investigators have worked with Warburg technique. It might be that the dense packing of the eggs and the shaking conditions in this procedure support the growth of bacteria, whereas the Cartesian diver technique is more favorable in this respect. However, in this investigation no particular measures have been taken against bacterial contamination.

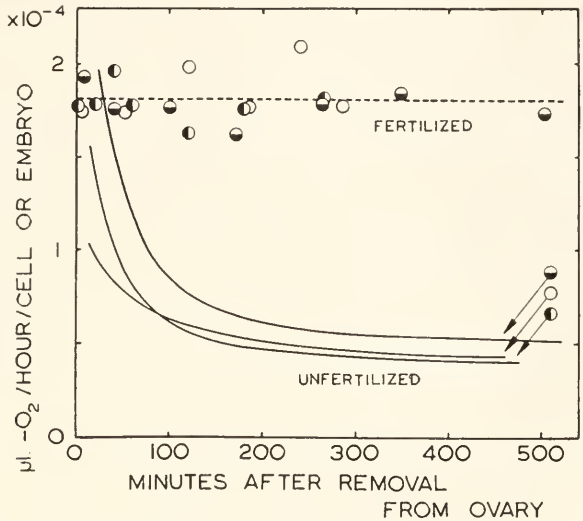

FIGURE 1. Oxygen consumption of unfertilized eggs and of fertilized eggs, fertilized at different times after the eggs' removal from the ovary. *Psammechinus miliaris.*

Each dot represents the respiration rate at 120 mins. after fertilization and is marked at the time of fertilization. ○, ◒ and ◐ stand for the three females used. Temperature 18° C. Oxygen consumption rate of unfertilized eggs at 230 mins. after removal from ovary: ○ = 0.50, ◒ = 0.58 and ◐ = 0.47 × 10^{-4} µl./cell and hour (mean value found by Borei 1948a = 0.53); mean value of rates of fertilized eggs: ○ = 1.85, ◒ = 1.78 and ◐ = 1.79 × 10^{-4} µl./embryo and hour (Borei, 1948a = 1.84). For the respiration of fertilized eggs, the best fit according to the method of least squares is indicated by a dotted line.

Borei (1948a, Chapter 3.21) compares the quotient between respiration after fertilization and that before, and finds that the values from different sea-urchin species vary considerably, and moreover that for one and the same species, greatly differing values are reported. So for example for *Arbacia punctulata* quotients from 2.6 to 5.3 have been given. For Psammechinus the values 3.6 and 5.7 are recorded. On the other hand, if the conditions of measurement are well defined as to time from fertilization and from removal from ovary respectively, the quotient will become fairly constant, as the results of Borei (1948a, Chapters 3.112.2 and 3.21) show. Considering the possible influence of values of pre-fertilization respiration on the quotient, obtained at different points on the declining egg respiration curve, Borei (1948a) thinks that quotient values given in the literature are of minor importance for quantitative considerations concerning respiration changes at fertili-

zation, but merely show that the oxygen consumption of the unfertilized egg some few hours after its removal from the ovary is considerably lower than that of the fertilized egg some few hours after fertilization. The present investigation supports this concept thoroughly. If the quotients from the experiments in Figure 1 are recorded (Table I), it is clearly seen that the quotient value will become smaller and smaller the closer the time of fertilization lies to that of egg removal. It would rather seem that values < 1.0 could be obtained in the earliest experiments, i.e. that the respiration of the just removed egg is actually higher than that of the fertilized egg during the first hours of development (cf. Borei, 1948a).

TABLE I

Quotients between oxygen consumption rate 120 mins. after fertilization and oxygen consumption rate of unfertilized eggs at the moment of fertilization. Psammechinus miliaris

Same experiments and denotations as in Figure 1. Value given by Borei (1948a) for 230 mins. after egg removal from ovary = 3.6. In quotients marked * respiration values of unfertilized eggs are obtained graphically from extrapolated curves.

○		◒		◐	
Mins. after egg removal	Quotient	Mins. after egg removal	Quotient	Mins. after egg removal	Quotient
6	<1.6*	8	<1.0*	2	<1.0*
52	2.3	40	1.2	21	1.2
120	3.3	100	2.2	41	2.5
185	3.5	170	2.5	60	2.8
241	4.3	264	3.2	120	3.1
286	3.8	349	3.5	179	3.6
		502	3.4	265	3.9

It would appear from the presented data, as the value of the pre-fertilization respiration rate seems to be of no importance for the oxygen consumption rate after fertilization, either that pre-fertilization respiration constitutes no integral part of the respiration after fertilization, or that fertilization brings about a release from inhibiting factors active on respiration in the unfertilized egg. As the oxidase system is probably the same both before and after fertilization (Runnström, 1930, Robbie, 1946; see also Introduction of the present paper), it is reasonable to suppose that changes occur at fertilization in those parts of the system which are situated between the oxidase and the dehydrogenases. The dehydrogenases themselves, however, are not likely to be affected. (Dimethyl-p-phenylenediamine experiments by Runnström, 1930 and 1932, Örström, 1932, Borei and Renvall, 1949; hydroquinone, Runnström, 1930; pyocyanine, Runnström, 1935; methylene blue, Runnström, 1930; cf., however, Ballentine, 1940, who claims that the dehydrogenases are not capable of maximum activity in the unfertilized egg.) (Cf. Korr, 1939: "release of substrate from precursors.")

From experiments on the respiratory quotient of pre- and post-fertilization respiration in the sea-urchin egg, it appears very probable that different substrates are utilized before and after fertilization. These changes in RQ upon fertilization,

which support the view that post-fertilization respiration competes with that of pre-fertilization, eventually suppressing it more or less completely, are seen from Table II.

It must, however, be kept in mind that a constant respiration part is assumed for the unfertilized egg (Borei, 1948a, Chapter 3.112.1) as well as a decreasing part. The above-presented data do not indicate whether even the constant respiration part is abolished at fertilization. It is still an open question whether this part of pre-fertilization respiration survives fertilization or not.

TABLE II

RQ of sea-urchin egg respiration before and after fertilization

Time in relation to fertilization	RQ	Species	Author
Before*	1–1.2		Borei (1934)
0–30 mins. after	0.84		Laser and Rothschild (1939)
30–40 mins. after 40–50 mins. after	0.78 0.64	*Psammechinus*	Borei (1934)
35–50 mins. after	0.66		Laser and Rothschild (1939)
1–2 hr. after 7–8 hr. after	0.73 0.85	*Paracentrotus*	Öhman (1940)
2-cell stage-hatching	0.8		Ephrussi (1933)

* Ashbel (1930) finds the value 1.06 before fertilization (Arbacia).

SUMMARY

Using Cartesian diver micro-respiration technique, it was found that in *Psammechinus miliaris* the rate of respiration of the newly fertilized egg is independent of the rate of respiration of the unfertilized egg at the moment of fertilization.

The quotient (respiration after fertilization)/(respiration before fertilization) was found to decrease considerably (probably even to values < 1.0) if the time interval between egg removal from the ovary and fertilization was diminished. If the decreasing part of pre-fertilization respiration is given time to disappear before fertilization, the quotient lies between 3 and 4.

It is thought possible that the decreasing respiration part of the unfertilized egg is abolished upon fertilization, due to probable changes in the function of members of the oxidizing system, situated between the oxidase and the dehydrogenases. It cannot be decided from the experiments whether the constant respiration part of the unfertilized egg still participates in the respiration of the fertilized egg or not.

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